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# Separation Materials: Proteins make for finer filters

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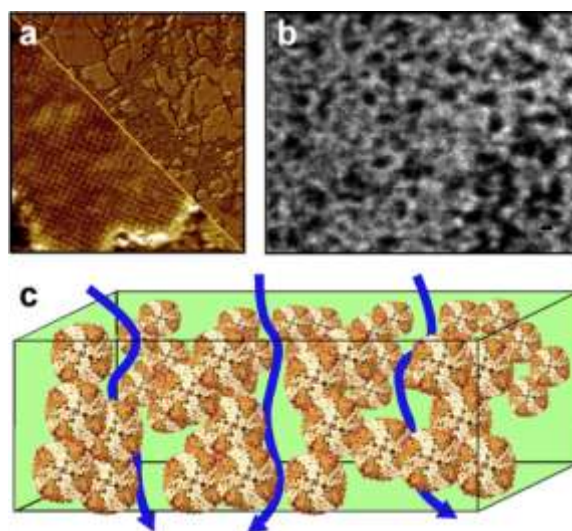
# Fast Flow Through Ferritin Nanogaps

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Membrane interfaces are unsung heroes of modern world: they permeate its every aspect, yet they rarely get the spotlight. The basic concept is deceptively simple: a membrane barrier lets one kind of species go through and keeps another kind from passing to the other side. Membranes provide one of the most energy-efficient ways to separate chemical species based on size, charge, or chemical properties. Modern industrial membrane technology, dominated by polymer membranes, has made impressive achievements ranging from the wide availability of clean safe water to the development of efficient hemodialysis procedures. Multibillion-dollar annual membrane market is perhaps the best testimony to commercial importance of membranes, yet the technology can still use many improvements. In this issue Ichinose and colleagues report a membrane fabrication method that bypasses polymers in favor of an approach based on templated self-assembly of biological building blocks.

It is no surprise that biology serves as inspiration for membrane development. Biological membranes enable living systems to function without rapidly descending into the state of entropically-driven chaos. Cells use a dizzying array of sophisticated pores that range from passive channels such as aquaporins in kidneys, to sophisticated ion pumps and ion channels in heart tissue. The simplest and yet one of the most elegant example of a biological membrane is the S-layer that some bacteria use to protect their outermost walls (Fig 1a). This crystalline layer, composed of a single glycoprotein, covers the cell surface and provides a defined porosity barrier between the bacteria and the outside world. The 2-3 nm size pores formed by the adjacent proteins in the S-layers provide ample room for the nutrients and metabolites to pass back and forth, and still protects the bacteria from enzymes and phages that threaten its existence. Crystalline nature of the S-layers ensures remarkable uniformity of its pores; a feature that inspired Sara and Sleytr<sup>1</sup> to separate the S-layer from the bacteria, place it on a solid support and then cross-link the proteins to create the first biologically-



**Figure 1.** **a.** AFM images of the S-layer proteins at low (right) and high (left) resolution (from: [www.veeco.com](http://www.veeco.com)). **b.** TEM micrograph of a PVDF-g-PEGM membrane. Bright areas indicate PVDF domains (from ref 4). **c.** Schematics of a membrane layer made from cross-linked ferritin subunits (see paper by Ichinose and colleagues in this issue).

derived ultrafiltration membrane. Remarkably, even today the S-layer membranes are remarkable performers: they are stable over a wide pH solution range and their size rejection curve is unusually steep relative to the rejection characteristics of polymer-based membranes.

Unfortunately, scaled-up production of biologically-derived membranes proved to be a formidable challenge, which led researchers and the membrane industry to focus on making uniform pore barrier layer membranes using synthetic building blocks<sup>2</sup>. Some of the most successful strategies in these area use self-assembly of block copolymer templates to generate a regular lamellar structure and then to convert it into a membrane by removing one of the polymer components. Hashimoto and colleagues first demonstrated this process by synthesizing a microdomain-separated polystyrene-polyisoprene (PS-PI) film and then degrading the PI component with ozonolysis, which left them with channels in the film<sup>3</sup>. Further refinements, such as use of triblock copolymers have increased the system tunability and control. Mayes and colleagues demonstrated that a similar concept could be translated into a high-performance membrane by using a PVDF-grafted-poly(PEGmetacrylate) system (Fig 1b) cast on an ultrafiltration membrane support<sup>4</sup>. The membrane obtained by this process showed high fluxes on the order of 9 L/hm<sup>2</sup>bar.

On page XX of this issue, Ichinose and colleagues synthesize these concepts in an experiment that uses self-assembly of globular proteins on sacrificial nanofilament templates to produce an extremely permeable and robust membrane (Fig. 1c) with rejection properties similar to low MW cutoff ultrafiltration membranes. This fabrication technique is remarkably versatile, as it can use many different globular protein building blocks. Ichinose group chose ferritin because it produced membranes with very high water permeance. To make this membrane researchers first organized ferritins on cadmium hydroxide nanostrands and then assembled them into a filter cake. After cross linking the proteins they removed the metal hydroxide, leaving an extremely chemically-resistant 60 nm thick membrane stable in organic solvents and in a wide range of pH (1.5 to 13). Remarkably this membrane showed pore sizes smaller than 2.2 nm and water permeance of up to 9000 L/hm<sup>2</sup>bar.

Ichinose and colleagues also explored rejection properties and mechanisms of ferritin membranes using a wide range of analytes and solution conditions. Although more experiments will be needed to completely characterize the MW cutoff and to fully understand the rejection mechanisms in all their complexity, the presented data shows several trends. First, it is clear that pH changes can modulate the pore size to some extent. Second, near complete rejection of square-planar TMPy porphyrin (MW ~680) and low rejection of linear polyelectrolyte PSS (MW ~70000) demonstrated the strong dependence of the rejection on the shape of the molecule. It is possible that further engineering of ferritin or other protein building blocks could enable further fine-tuning of membrane cutoff and rejection properties.

Finally, the most remarkable and commercially attractive property of ferritin-based membrane is its combination of high permeance and small pore size. Measured water permeance of 9000 L/hm<sup>2</sup>bar is comparable to commercial

ultrafiltration membranes that have MW cutoffs in the range of 1,000KDa (Millipore Biomax 1000). Permeances of commercial membranes with MW cutoffs of ~3KDa, that are comparable to those of ferritin protein membranes, are more than two orders of magnitude lower! Researchers attribute such low protein membrane impedance to a short— less than 6nm-long— nanopore formed between ferritin molecules.

This new class of protein membranes could potentially be a very attractive replacement for some of the conventional UF and NF membranes. Enhanced chemical stability may also open new opportunities for separations in chemical industry. Yet, it remains to be seen whether these protein membranes will be easier to manufacture than their S-layer membrane predecessors, since only a cost-effective manufacturing process will realize the full potential of these membranes in the marketplace.

#### **References:**

- <sup>1</sup> Sara, M. & Sleytr, U., Production and characteristics of ultrafiltration membranes with uniform pores from two-dimensional arrays of proteins. *J. Membr. Sci* 33, 27-49 (1987).
- <sup>2</sup> Ulbricht, M., Advanced functional polymer membranes. *Polymer* 47 (7), 2217-2262 (2006).
- <sup>3</sup> Hashimoto, T., Tsutsumi, K., & Funaki, Y., Nanoprocessing Based on Bicontinuous Microdomains of Block Copolymers: Nanochannels Coated with Metals. *Langmuir* 13 (26), 6869-6872 (1997).
- <sup>4</sup> Akthakul, A., Salinaro, R., & Mayes, A., Antifouling polymer membranes with subnanometer size selectivity. *Macromolecules* 37 (20), 7663-7668 (2004).

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